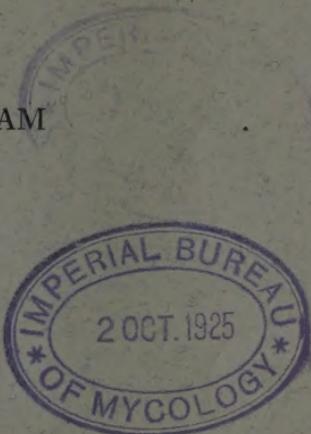


THE STRUCTURE AND DEVELOPMENT
OF TWO NEW ZEALAND SPECIES
OF SECOTIUM

BY

G. H. CUNNINGHAM



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THE STRUCTURE AND DEVELOPMENT OF TWO NEW ZEALAND SPECIES OF SECOTIUM.

(With Plates XI and XII.)

By G. H. Cunningham,
Government Mycologist, Wellington, N.Z.

IN the course of a revision of the Australian and New Zealand species of the genus *Secotium* (1924) the writer was somewhat doubtful as to the position this genus should occupy among the Gasteromycetes. The earlier taxonomists placed it in the tribe Podaxineae of the family Lycoperdaceae, a classification generally followed; Fischer (1900) proposed the family Secotiaceae to include it and three other genera, and more recently, Conard (1915), as a result of the study of the development of *S. agaricoides* (Czern.) Hollós, has suggested that it should be placed in the Agaricaceae. As *S. agaricoides* is a somewhat aberrant species of the genus, and as several of Conard's conclusions are at variance with ideas held by the writer a search was made for developmental stages of two of our most common species. By thoroughly working out the development of these two species, it was hoped that some light might be shed on the taxonomic position of the genus.

Two species were chosen, one, *Secotium erythrocephalum* Tul. being typical of the genus as now defined, the other, *S. novae-zelandiae* G. H. Cunn. (*loc. cit.*, p. 11) being, in the structure of the mature plant, intermediate in position between *S. erythrocephalum* and *S. agaricoides*.

Although the following details (with minor differences) are equally applicable to either species, *S. novae-zelandiae* is discussed at greater length, as more abundant developmental stages of this species were obtained.

The genus *Secotium* is characterised by the fact that the coriaceous peridium encloses a cellular gleba, and is borne on a definite central stipe which continues through the gleba as a columella and merges with the apex of the peridium. Dehiscence is supposed to be effected by the tearing apart of the margin of the peridium from the stipe, but as this does not expose more than a very minute portion of the hymenium, and as it very frequently does not occur at all in different plants of the same species (figs. 13, 18), it would appear that the plants may be more correctly considered to be indehiscent.

Secotium novae-zelandiae* (fig. 5).*MATURE PLANT.**

The *peridium* is usually ovate in shape, and may attain a size of 3-5 cm. high by 1.5-3 cm. broad. It is french-grey in colour, smooth, coriaceous and slightly viscid. It is about 3 mm. thick at the apex where it merges into the columella, tapering to the base where it is lacerate and somewhat folded. The folds are commonly decurrent and pressed to the stipe. Sometimes they are held in position by remnants of the partial veil. The peridium consists of a single layer, composed of closely-compacted hyphae, whose long axes are predominantly radial.

The *gleba* is sepia-coloured and is formed of numerous anastomosing tramal plates enclosing lacunae; these are either quite large and irregular in shape, the septa being gill-like, or else definitely cellular. Specimens with a gill-like gleba approach *S. agaricoides*, and those with a cellular gleba, *S. erythrocephalum*; but the prominent stipe, smooth cortex and large elliptical spores connect it with the latter species. Extreme types of this species, as well as intermediate connecting forms, may be collected from the same substratum, where they may appear growing side by side.

A tramal plate consists of the following three layers: (a) the trama, consisting of a central layer of septate hyphae arranged in a parallel manner, with the long axes of the cells parallel to the surface of the plate (fig. 1, *tr.*); (b) the subhymenium, a layer on either side of this consisting of numerous somewhat polygonal cells (fig. 1, *sub.*); and (c) the hymenium, an outer palisade layer of basidia, whose long axes are at right angles to the plate (fig. 1, *hy.*). On the basidia the spores, usually four in number, are borne on long and slender sterigmata. The spores are smooth, elliptic-ovate, and sepia in colour, the colour being confined to the wall. There are no paraphyses, cystidia or other aberrant cells.

The *stipe* is up to 4 cm. long, by 4-6 mm. thick, hollow, and somewhat fibrillose on the exterior; the base is attached to the substratum (decaying wood) by numerous yellow- or violet-tinted rhizoids. The stipe continues to the apex as the *columella* —the basal portion of which is free and surrounded by a narrow conical cavity, but its upper half is attached to the tramal plates of the gleba. No trabeculae are present. Spore dispersion is dependent upon the decay of the plant, or, as this species is readily eaten by slugs, it is probable that these animals serve in some manner to disseminate them.

Both this and the following species grow upon decaying wood; *S. novae-zelandiae* is confined to lowland rain-forest, but *S. erythrocephalum* is not uncommon in gardens, especially where the soil has been at one time in forest.

DEVELOPMENT.

General.

In all some twenty-five developmental stages of this species have been collected; of these fourteen were sufficient to give a connected idea of development.

All were fixed in picro-formol (which gave the most satisfactory results of all the solutions tried), and stained with iron-alum haematoxylin, followed by 1 per cent. iodine-green in clove oil; this combination gave excellent results, much more satisfactory than numerous others that were experimented with.

As the plants are gregarious, it is not unusual to find many developmental stages attached to the same rhizoids on one piece of decaying wood. The young plants are first noticeable as small white swellings on the upper portion of a rhizoid, often close to a more mature plant. Sections show these to consist of closely-woven undifferentiated hyphae (fig. 6), exactly resembling in shape and size those constituting the rhizoid. The young plant begins to elongate, until it is about twice as long as broad, being at this stage about 2×1 mm. Then a small radial indentation appears on the exterior, marking off the peridium from the stipe; this is followed by somewhat rapid growth in diameter of the peridium, which gradually increases in size until it becomes two or three times the diameter of the stipe. The peridium also assumes a definite shape, for in quarter-grown specimens it has the form of the mature plant. It continues to enlarge until about three-fourths the size of the mature plant, when the stipe begins rapidly to elongate, carrying the mature peridium upwards until it stands some 25 mm. or more above the substratum. At first the young plant is dingy-grey, but when about one-third grown, the french-grey colour begins to appear in the peridium and stipe; and in old specimens the colour changes to cyano-blue or some shade of green.

Development of the peridium and gleba.

When the plants are about 2 mm. long sections show that differentiation of the peridium and stipe has commenced. The stipe is the first to become differentiated, and at this stage consists of hyphae arranged in parallel fashion, with their long axes parallel to the long axis of the plant. Near the apex these hyphae become merged with closely and intricately-woven hyphae, the primordium of the gleba and peridium. This area becomes further differentiated, and a ring of deeply-staining tissue appears near its base. It may be seen closely appressed to and completely surrounding the columella, which at this stage is well developed at the base, although it has not become

differentiated at the apex. There is as yet no indication of the presence of the peridium, nor is any universal veil present in this or any succeeding stage. In this deeply-staining ring a small radial lacuna appears, and below it, running downwards and outwards from the inner and lower margin of the columella to the margin of the apex of the stipe, appears a wedge-shaped radial ring of loosely-woven hyphae (fig. 7). This is the first appearance of the partial veil, the development of which is discussed in detail later. This lacuna, which extends in a ring around the base of the columella, enlarges, and the hyphae lining its roof become arranged in a palisade manner. These hyphae are thinner than those of the stipe, contain abundant protoplasmic contents, and are closely compacted together. At first they are confined to the roof, but soon extend around the walls until the whole cavity, with the exception of the basal portion, or floor, is lined with them. The cavity next alters in shape, becoming laterally compressed, and extends further into the upper part of the as yet undifferentiated portion of the gleba. Into it, downward growths of the palisade layer begin to penetrate (fig. 8); these grow until they come in contact with one of the lateral walls, with which they merge, thus dividing the original cavity into several smaller ones. At the same time further lacunae begin to form in the undifferentiated portion of the gleba immediately above the original cavity. Spores now appear on a few of the first formed palisade cells (fig. 9) not as yet differentiated into regular basidia, for no definite sterigmata are present, the spores being borne singly on terminal projections. Further septa continue to grow from the roof of the original cavity and merge with its side walls, and numerous lacunae are at this stage present in the upper portion of the peridium. These continue to form until the whole of the gleba is divided into numerous cellular areas. Basidia bearing the normal number (four) of spores now appear on the first-formed tissue. The lower portion of the gleba begins to separate from the base of the columella, and this continues until a small cavity, conical in shape, is formed around the lower half of the columella. Next, the peridium becomes differentiated; at first it is quite thick, save at its margin, but it soon becomes thinner, owing to the appearance of numerous lacunae in the hyphae bordering its inner surface. From this stage until maturity, development of the gleba consists in the formation of further lacunae in the thick tramal plates (figs. 11, 12).

These lacunae appear in the following manner: cells of certain hyphae in a definite area of the tramal plate become slightly inflated, and numerous septa appear in them; this is followed by the tearing apart of these hyphae so that a small cavity is

formed (fig. 12). This increases in size and the elements of the hymenium become differentiated, so that a portion of the original plate is split into two, and is consequently thinner than before. Increase in the surface of the plates occurs, and as a result the plates are thrown into numerous folds, which may at different points come in contact with other plates and anastomose. Fusion is effected as follows: the spores are first crowded to one side as the plates approach one another, then the basidia become somewhat compressed and crushed to one side; from the subhymenium small cells grow out and merge with those of the opposite plate.

Owing to the method of the formation of these lacunae the cavities in the mature plant are very numerous, and much smaller in size than in the young plant.

From the time plants are about half grown until they reach maturity spores are being produced in ever increasing numbers; indeed, so abundant is their production that small lacunae may be filled with them, and commonly they are two or more layers deep in all the lacunae of the mature plant.

Development of the stipe and columella.

The stipe becomes differentiated immediately before the primordium of the gleba. It then continues to grow until about the time of the formation of the first cavity of the gleba, when the cavity of the stipe makes its appearance (fig. 9). The stipe then makes little growth until the plant is about one-third grown, when it increases first in thickness, and then begins to elongate rapidly until it attains full size.

The columella is discernible immediately before the appearance of the first cavity, and extends for a short distance into the as yet undifferentiated gleba. It then continues to develop slowly until the time of the appearance of the first few septa in the glebal cavity, when it is seen to have extended almost to the apex of the peridium. It does not merge with the peridium until the plant is about half grown, for the latter structure is not discernible until the glebal cavities are somewhat numerous, being represented at first only by loosely-woven hyphae.

Development of the velum partiale and mode of "dehiscence."

The first indication of a partial veil has been previously discussed. It appears immediately after the first glebal cavity and may at this stage be seen as a wedge-shaped radial area running downwards and outwards from the base of the cavity to the outer and upper portion of the stipe. It is first noticeable owing to the presence of numerous air spaces between the hyphae of this region, these hyphae being more loosely woven than those

of the primordium of the gleba. These spaces gradually increase in size, and as the peridium and stipe develop, become separated more and more, in many cases being torn apart, until at maturity a few hyphae only are present, attaching the base of the peridium to the stipe. Once the partial veil becomes differentiated it would appear that little if any further growth takes place in this region, so that the separation of the hyphae and the appearance of the large air spaces results entirely through the further growth of the stipe and peridium.

Remnants of the veil persist on the periphery of the stipe and give to it a somewhat fibrillose appearance. These remnants are accounted for when the development of the stipe is considered, for when it is about 2 mm. long the partial veil is present, attached to and surrounding it throughout its length (fig. 9). As the stipe elongates, the fibrils of the partial veil are more and more widely separated until they are torn away from the upper points of attachment, when the remnants persist on the periphery of the stipe as the fibrils alluded to.

"Dehiscence" as a rule does not proceed further than this, for one frequently obtains mature plants in which the margin of the peridium is firmly united to the stipe by these remaining fibres of the partial veil.

Development of *Secotium erythrocephalum*.

Early stages of this species show characters similar to those of the preceding save that the first glebal cavity appears immediately before differentiation of the columella has begun. Later stages also are similar, save that the stipe becomes more thickened and does not elongate to the same extent (fig. 16); then, too, the lacunae appear more frequently and are much smaller in size, and the trama plates are thinner.

The peridium early becomes covered with a definite gelatinous layer, formed of hyphae which have become gelatinised (fig. 17). The colour of the peridium appears when the plants are about one-quarter grown. The first few layers of the hyphae forming the peridium, underlying the gelatinous layer, become partially filled with granules of some pigment which readily take the haematoxylin stain. They appear to be imbedded in the protoplasm lining the hyphal walls, and become more plentiful as the plant approaches maturity. This layer is absent in the preceding species.

CYTOTOLOGY.

Cytological details appear to be the same in both species. The hyphae of the columella, stipe and peridium are invariably binucleate, the nuclei being close together and usually side by

side. Cells of the hyphae of these tissues are from $40-60\mu$ long \times $8-10\mu$ thick; the basidia are $15-25 \times 5-8\mu$. The basidia at first are binucleate; the two nuclei fuse, and a somewhat larger fusion nucleus is formed; this takes up a position near the free end of the basidium, and then divides twice, the first division preceding the formation of sterigmata, the second following their appearance. After the sterigmata have begun to elongate the spores appear, and are about half size when the sterigmata have attained their full size, $8-15\mu$. A nucleus migrates into each spore when they are about one-quarter size, for it is not present in spores smaller than this, although invariably present in spores that are larger (fig. 2). This nucleus divides mitotically when the spore is about half size, so the mature spore is binucleate (figs. 1, 2), a character difficult to determine, as at this stage the nucleus does not readily take the stain. The nuclei are highly refractive bodies, are exceedingly small, $2-5\mu$, and contain one large readily-staining nucleolus. The spore eventually attains full size, and begins to colour, becoming finally sepia, the colour being confined to the episporule.

Clamp connections are abundant in the tissues of the stipe and the hyphae constituting the partial veil (fig. 3).

The writer has grown specimens of *S. novae-zelandiae* on rotting twigs buried in leaf-mould in the laboratory, and finds that development is a slow process; from the time of the beginning of differentiation until maturity covers a period of from two to three months. Where insects are excluded the plant survives for at least a month after reaching full size, then it wilts and finally deliquesces, this action being probably hastened by bacteria, as these organisms are abundant in old specimens.

CONSIDERATIONS REGARDING THE TAXONOMIC POSITION OF THE GENUS.

From a consideration of the facts set out above, it is seen that until the appearance of the first cavity the development of the two species is somewhat similar to that of *Agaricus Rodmani*, as recorded by Atkinson (1915); but from this stage onwards the development has little in common with that of *Agaricus*, or in fact with that recorded of any member of the Hymenomycetes, but approaches closely that of certain genera of the Hymenogastrineae. In particular, the repeated appearance of fresh lacunae in the undifferentiated portions of the gleba, followed later by the appearance of these spaces in the trama plates, is in close agreement with the development of the gleba in certain genera of this family. Further, the indehiscent nature of the peridium, the cellular structure of the gleba, the basidial characters and copious spore production are

typically gasteromycetous characters. In fact, were these plants devoid of columella and stipe, they would be placed in the Hymenogastrineae without hesitation. Taking these facts into consideration the writer believes that the genus must be retained in the Gasteromycetes.

On account of the structure of the gleba, the nature of the basidia and spores, and the presence of a definite stipe and columella, the genus forms a well-defined group. The presence of the stipe and columella, together with the similarity of the early developmental stages, would tend to link it with the Agaricaceae, whereas the nature of the gleba and peridium, together with the later developmental characters, link it with certain genera of the Hymenogastraceae. It therefore occupies an intermediate position, and as no genera are known connecting it with either family it should be retained in a distinct family. The Secotiaceae of Fischer (1900) will, however, have to be emended to include only those genera possessing a stipe, columella, cellular gleba, tetrasporous sterigmatic basidia, those with a capillitium being excluded. As the structure and development of the other genera which Fischer has included in the Secotiaceae are practically unknown, no opinion can be expressed concerning them.

From a consideration of the available literature it appears to the writer that the present unsatisfactory classification of the Gasteromycetes can be attributed to: (a) little or no consideration having been taken of developmental characters, (b) genera and species having been based almost solely on morphological (macroscopic) characters, and (c) the microscopic structure of the basidium, spores and other characters having been completely ignored. It is hoped to investigate the development of all the genera of New Zealand Gasteromycetes with the view that ultimately sufficient information will be collected to place the classification of this sub-class on a more satisfactory basis.

The writer wishes to acknowledge the assistance of Mr J. C. Neill of this laboratory for aid in collecting and for preparing the sections of the developmental stages of both species.

SUMMARY.

1. The development of two species, *Secotium erythrocephalum* Tul. and *S. novae-zelandiae* G. H. Cunn., is dealt with.
2. Differentiation of the stipe and columella precedes the formation of the first glebal cavity in *S. novae-zelandiae*, and follows its appearance in *S. erythrocephalum*.
3. Following the appearance of the first glebal cavity, the gleba of both species becomes further differentiated through the appearance of numerous lacunae in the primordium of the gleba surrounding the columella.

4. Spores make their appearance shortly after the formation of the first few cavities, when the plant is less than one-third the normal size.

5. A definite partial veil is present, but a universal veil is wanting.

6. Early developmental stages, until the appearance of the first glebal cavity, resemble those of *Agaricus*, later stages resemble certain genera of the Hymenogastraceae.

7. Spores and hyphae are binucleate.

8. The genus should be retained in the Gasteromycetes, preferably in a distinct family, the Secotiaceae (emended).

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EXPLANATION OF PLATES XI AND XII.

[The drawings have been made with the aid of a camera lucida.]

Fig. 1. Section of a trama plate of *Secotium erythrocephalum* Tul. *Tr.* = trama; *sub.* = subhymenium; *hy.* = hymenium. $\times 1000$.
 Fig. 2. Basidia of *S. novae-zelandiae* G. H. Cunn. $\times 1000$. Showing various stages of nuclear division and spore formation. Fifth basidium (from left) shows the original binucleate condition, the second basidium shows these nuclei before fusion, the fourth the fusion nucleus; the sixth basidium shows four nuclei prior to migration into the spores, the first basidium shows the spores, each with a single nucleus; the third basidium bears four binucleate spores.
 Fig. 3. Clamp connections from hyphae of the partial veil. $\times 1000$.
 Fig. 4. Photograph of *S. erythrocephalum*. Natural size.
 Fig. 5. Photograph of *S. novae-zelandiae*. Natural size.
S. novae-zelandiae.
 Fig. 6. Undifferentiated primordium. $\times 30$.
 Fig. 7. First appearance of the radial cavity. $\times 30$.
 Fig. 8. The radial cavity has enlarged and is becoming divided into smaller areas by downgrowths from the roof of the cavity. $\times 25$.
 Fig. 9. Young plant, showing greatly elongated stipe, well-defined partial veil and cavity of the stipe. $\times 20$.
 Figs. 10-12. Progressive development of the peridium and gleba. $\times 10$.
 Fig. 13. Not quite median section through a mature plant (lamellar form). $\times 4$. Note that the base of the peridium is still attached to the stipe by remnants of the partial veil. The stipe is cut at an angle, hence the cavity is not shown in the section. Perforations in the stipe are due to insect injuries.
S. erythrocephalum.
 Fig. 14. Plant after first septa have been formed in the first glebal cavity. (The earlier stages are similar to the preceding, so are not shown.) $\times 30$.
 Fig. 15. Later stage in which numerous plates have appeared. Note the well-developed partial veil. $\times 25$.
 Fig. 16. Later stage showing further development of the gleba. $\times 15$.
 Fig. 17. Section showing the tremendous development of the gleba. $\times 8$.
 Fig. 18. Not quite median section through mature plant (small form). Note that the base of the peridium is attached to the stipe. The cavity of the stipe is not apparent, owing to the stipe being cut at an angle. Perforations in the stipe are due to insect injuries.

